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Sensitive MS/MS–liquid chromatography assay for simultaneous determination of tegafur, 5-fluorouracil and 5-fluorodihydrouracil in plasma

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Abstract

Ftorafur (FT), an oral prodrug of 5-FU, is part of UFT and S1, two oral prodrugs widely used in digestive tract cancer. We set up a liquid chromatography tandem mass spectrometry (LC/MS–MS) method, chosen for its specificity of detection, for simultaneously measuring in human plasma FT, 5-FU and 5-FUH_2. Separation was performed on a Hypercarb column. Linearity, precision and accuracy were validated in the concentration range studied for each compound. This simple and reliable LC/MS–MS method allows specific, sensitive and reproducible quantification of FT, 5-FU and FUH_2 in human plasma and can be applied to further pharmacokinetic studies in patients treated with FT-based prodrugs.

Keywords: Tégafur; UFT; S1; 5-Fluorouracil; 5-Fluorodihydrouracil; LC/MS–MS

1. Introduction

Ftorafur (FT), 2-tetrahydrofuranyl derivative of 5-FU, is a prodrug of 5-FU and as such is part of two oral drugs, UFT and S1. FT is converted to 5-FU by certain hepatic microsomal cytochrome P450 (CYP450) enzymes, or by ubiquitous cytosolic enzymes [1,2]. It then follows the same metabolism pathway as 5-FU, which first catabolism step is the reduction to 5-fluoro-5,6-dihydrouracil (FUH_2) by dihydropyrimidine dehydrogenase (DPD).

The main enzyme responsible for FT transformation to 5-FU is CYP2A6. The CYP2A6 activity has first been studied in coumarin and nicotine metabolism and has been shown to exhibit wide interindividual variability [3–5]. This variability has been attributed to a genetic polymorphism of CYP2A6, with more than 10 different alleles reported [6–10]. Moreover, two mutant alleles have been found in a cancer patient presenting increased level of FT after administration of a FT based treatment [11]. Thus, evaluation of CYP2A6 activity in cancer patient seems essential for optimizing the treatment efficacy and could be calculated by the 5-FU/FT plasma ratio.

Moreover, DPD activity varies widely between patients because of a genetic polymorphism too [12–14]. About 3–5% of the population present a major deficiency in DPD activity and are likely to undergo toxicity after treatment with fluoropyrimidines. Thus, evaluation of DPD activity by calculating FUH_2/5-FU plasma ratio complementary to 5-FU/FT ratio would be very helpful to optimize the treatment efficacy without increasing toxicity.

Several methods have been developed in an attempt to evaluate FT and 5-FU plasma concentrations. Most of them are complicated because of the use of two different analytical systems: high-performance liquid chromatography (HPLC) for FT and gas chromatography–mass spectrometry (GC–MS) for 5-FU [15,16]. Moreover, some detection systems, such as flame-ionization or nitrogen-phosphorus-

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sensitive detectors, used after GC separation, are not currently used in pharmacokinetic laboratories [17,18]. Another drawback of GC use is the necessity of an additional step of derivatization before analysis [19,15,16]. Likewise, the use of HPLC with fluorescence detection needs a derivatization reaction of FT and 5-FU for these compounds to become fluorescent [20].

More recently, HPLC methods allowing the simultaneous detection of FT and 5-FU have been developed [21–23]. However, some of them were aimed to quantify the compounds in rat or dog plasma and have not been tested in humans [21,22]. Using these methods in human plasma may necessitate modifications of the chromatographic conditions because of the presence of endogenous compounds that could differ from one species to another. To date, one method has been described for the simultaneous determination of FT and 5-FU in human plasma by HPLC [23]. However, this method did not allow the simultaneous detection of FUH_2, the first 5-FU catabolism compound formed by reduction of 5-FU by DPD.

Therefore, we set up a simple and reliable method for simultaneously measuring in human plasma not only FT and 5-FU, but also FUH_2. Our purpose was to explore metabolism pathway of FT after UFT or S1 administration to optimize the treatment according to the patient's metabolic capacities. We used liquid chromatography tandem mass spectrometry (LC/MS–MS) method because of its specificity of detection, which is essential in complex matrices like plasma where numerous endogenous compounds can co-elute with FT, 5-FU or FUH_2.

2. Experimental

2.1. Chemicals

FT, 5-FU, 5-chlorouracil (CU) and 5-bromouracil (BU) were purchased from Sigma (Saint Quentin Fallavier, France). FUH_2 was obtained from Roche (Basel, Switzerland). Acetonitrile, ethyl acetate and isopropanol were of HPLC grade (VWR International, Pessac, France). The water used was of Milli-Q grade (Millipore, Molsheim, France) and was degassed with helium before use.

2.2. Sample extraction

This extraction procedure is derived from that used by Gamelin et al. for the extraction of 5-FU and has been improved [24]. First, 25 μL of internal standard (CU, 2.5 μg/mL) were added to 500 μL plasma samples and vortex-mixed. Blank human plasma for calibration were prepared in pooled normal human plasma from heparinazed whole blood which had been centrifugated at 3000 rpm for 10 min.

Plasma proteins were then precipitated with 600 mg ammonium sulfate. After vortex mixing for 1 min, 4 mL isopropanol-ethyl acetate (15:85, v/v) were added. The samples were gently mixed for 5 min in a rotatory stirrer (45 turns per min) and centrifuged for 15 min at $3500 \times g$. The supernatant was transferred to a glass tube and evaporated at 56 °C for 20 min under a stream of nitrogen. The dry extract was reconstituted with 200 μL of water and filtered through a 0.45-μm vinylidene polyfluorure membrane plate (Millipore, Saint Quentin en Yvelines, France) before injection onto the column. Volume injection was set at 40 μL for 5-FU and FUH_2, whereas it was only 10 μL for FT.

2.3. Instrumentation and chromatographic conditions

The HPLC system consisted of a Perkin-Elmer Series 200 autosampler and two Perkin-Elmer Series 200 micro pumps (Courtaboeuf, France). Two analytical columns, with their corresponding guard columns, were evaluated to achieve separation of the compounds: reverse phase column XTerra MS C18 (100 mm × 2.1 mm; 3.5 μm) purchased from Waters (Saint-Quentin-en-Yvelines, France) and porous graphitic carbon phase column Hypercarb (150 mm × 2.1 mm, 5 μm) purchased from ThermoElectron (Courtaboeuf, France). Mobile phase gradient, composed of acetonitrile and water, was optimized and differed according to the column tested. However, for both columns, mobile phase was delivered at a flow rate of 0.2 mL/min and was directed to the mass spectrometer probe without split.

The mass spectrometer was an API 2000 triple quadrupole from Applied Biosystems (Les Ulis, France) equipped with a TurboIonSpray source. Instrument parameters were optimized using a 10 μL/min infusion of a 10 μg/mL solution of FT, 5-FU or FUH_2 in methanol-water (50:50, v/v). The TurboIonSpray source was operated in negative ion mode with a needle voltage of −4500 V for FUH_2 and −4200 V for FT and 5-FU. The nebulizing gas was air delivered at 45 psi. The auxiliary gas was air at 70 psi for FUH_2 and 80 psi for FT and 5-FU and was heated at 400 °C. The collision gas was N_2 and the cell pressure was 3 mTorr. Collision energy was set at −28 eV for FUH_2 and −30 eV for the other compounds. The instrument was operated in multiple reaction monitoring (MRM) mode to detect the specific transition of precursor ion to fragment for each compound.

The acquired data were processed using the Analyst 1.1 software (Applied Biosystems).

2.4. Method validation

2.4.1. Linearity

FT, 5-FU, FUH_2 and CU were dissolved in Milli-Q water at a concentration of 1 mg/mL and stored at −20 °C. Standard solutions were prepared by further dilution of the appropriate compound into Milli-Q water. Calibration curves of 5-FU, FUH_2 and FT were prepared by adding 25 μL standard solution of the appropriate compound and 25 μL of the internal standard (CU) to 475 μL of control human plasma. The final generated concentrations were 5, 12.5, 25, 50, 125, 250, 500 ng/mL for 5-FU and 12.5, 25, 50, 125, 250,

500 ng/mL for FUH_2. For FT, because of the wide concentration range analyzed, calibration was achieved with two different curves: one for the lower concentrations (25, 50, 250, 500, 1000, 2500 ng/mL) and an other for the higher concentrations (2500, 5000, 10,000, 15,000, 25,000 ng/mL). Internal standard concentration was set at 125 ng/mL for lower concentration and 5000 ng/mL for higher ones. All the samples were then treated according to extraction and HPLC procedures.

Calibration graphs were obtained using the least-squares method. Standard curves for FT, 5-FU and FUH_2 were generated by plotting the peak area ratio of FT, 5-FU, or FUH_2 to that of the internal standard versus the concentration of each compound.

2.4.2. Limits of quantitation

The limits of quantitation (LOQ) were determined for FT, 5-FU and FUH_2. For each compound, LOQ was calculated as the minimum concentration that gave a relative standard deviation less than 10%.

2.4.3. Analytical recovery

Similar samples as those used for generating calibration curves were prepared ($n = 9$). Three different concentrations for each compound were studied, whereas concentration of CU was maintained constant. The recovery of FUH_2, 5-FU, FT and CU was evaluated by comparing peak areas obtained for these extracted samples to those obtained by direct injection of standard solutions of the same concentration.

2.4.4. Precision and accuracy

For the determination of within-day precision and accuracy, five samples of each concentration used to generate calibration curves were extracted and injected on the same day. For between-day precision and accuracy, one sample of each concentration was analyzed per day on five consecutive days.

3. Results

3.1. Internal standard

Two compounds, CU and BU, have been tested as internal standard. Their retention times, as well as that of FT, in the final chromatographic conditions were 12.1, 12.7 and 13.1 min, respectively. As all these compounds generated the same fragment ion at *m/z* 41.9, it was more advisable to select the internal standard presenting the more distant retention time compared to FT in order to avoid crosstalk. Thus, CU was at last selected as internal standard for the analysis.

3.2. MS–MS analysis

Chemical structures of Ftorafur, 5-FU, 5-FUH_2 and CU are presented in Fig. 1. Full scan mass spectrum of each compound is presented in Fig. 2. The acquisition was performed in negative ion mode via separate infusion at 10 μL/min of solutions of 10 μg/mL of each compound. The $[M–H]^-$ ions of FT, 5-FU, FUH_2 and CU analyzed in unit resolution were observed at *m/z* 199.0, 128.8, 130.8 and 144.8, respectively. In the CU spectrum, ion at *m/z* 146.8, which intensity is third of ion at *m/z* 144.8, was attributed to CU containing the chlorine stable isotope ^{37}Cl.

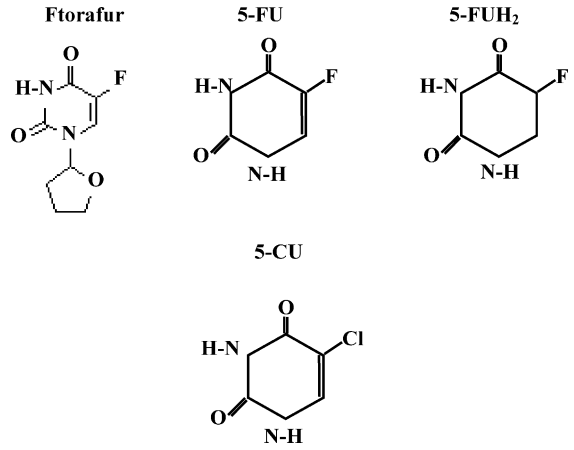

Fig. 1. Chemical structures of Tegafur (FT), 5-FU, 5-FUH_2 and CU.

After fragmentation in the collision cell, the $[M–H]^-$ ions of FT, 5-FU, FUH_2 and CU led all to the formation of the same product ion at *m/z* 41.9. The MRM transitions chosen for the quantitative experiments are summarized in Table 1.

3.3. HPLC procedure

3.3.1. Analytical column

Retention times and resolution of FT, 5-FU, FUH_2 and CU were evaluated for two different analytical columns. On reverse phase XTerra column, 5-FU, FUH_2 and CU are poorly retained, with retention times of 1.8 min for 5-FU and FUH_2 and 2.2 min for CU. This poor retention on traditional silica gel stationary phase, even when the mobile phase used is totally aqueous, can be explained by the high polarity of the compounds and results in laborious separation from other compounds present in plasma. Thus, a competition in the ionization step can happen between all the compounds, inducing a reduction of intensity.

The peculiar physical properties and retention mechanism of the Hypercarb column (stationary phase with a nonderivatized porous graphitic carbon surface) have allowed us to develop a method where FUH_2, 5-FU, FT and CU were

Table 1
MRM transitions chosen for the quantitative analysis of FT, 5-FU and FUH_2

	m/z precursor ion	*m/z* product ion
FT	199.0	41.9
5-FU	128.8	41.9
FUH_2	130.8	41.9
CU	144.8	41.9

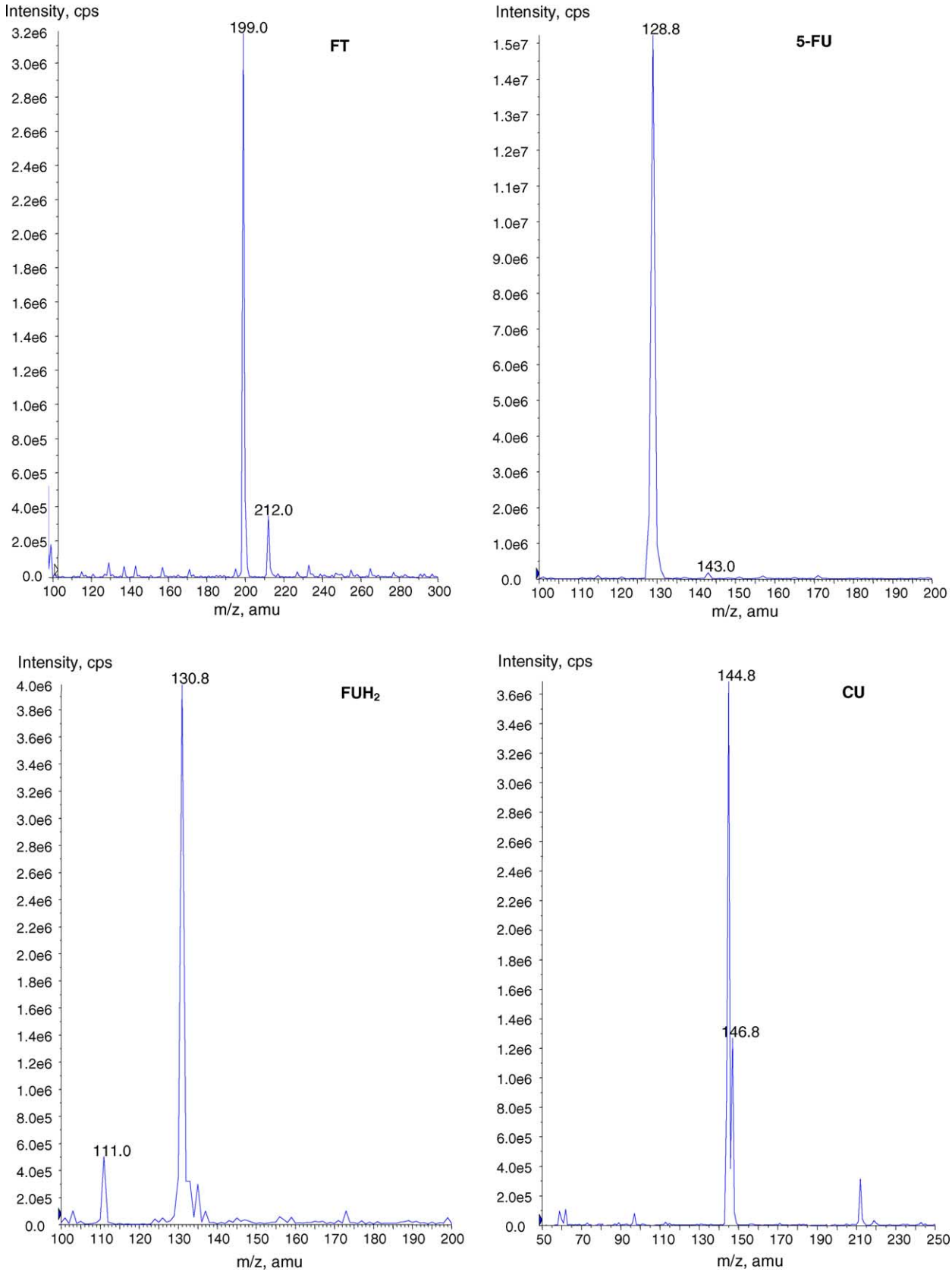

Fig. 2. Full scan mass spectrum of FT, 5-FU and FUH_2 in negative-ion mode resulting from direct infusion of a solution of 10 μg/mL of each compound (sum of 10 successive scans).

eluted at 3.9, 10.0, 13.1 and 12.1 min, respectively. Moreover, the mobile phase used with this column contained at least 12% organic solvent, which facilitates evaporation in the mass spectrometer source.

Given the large difference of retention time between FUH_2 and the three other compounds, chromatogram was divided in two periods. In the first one (0–6 min), only the transition corresponding to FUH_2 (130.8 → 41.9) was detected, allowing thus to obtain the maximum intensity for FUH_2 which was the analyte having the lowest sensitivity among the four compounds. In the second period, ranging from 6 to 20 min, mass spectrometer was set to detect the three transitions corresponding to 5-FU, FT and CU.

3.3.2. Elution gradient optimization

The elution gradient, consisting in water and acetonitrile, was optimized for the Hypercarb column maintained at ambient temperature. Elution was performed for a total run time of 20 min by applying a linear gradient as follow: 12% acetonitrile was applied for 2 min followed by an increase from 12 to 70% acetonitrile in 3 min; then a 70% acetonitrile phase was applied for 5 min followed by a linear gradient from 70 to 12% acetonitrile in 2 min; the column was at last equilibrated with 12% acetonitrile for 8 min before next analysis (Table 2).

Table 2
Elution gradient

Time (min)	0	2	5	10	12	20
ACN (mL)	12	12	70	70	12	12
Water (mL)	88	88	30	30	88	88

The first step at 12% acetonitrile was set to obtain a retention time of FUH_2 sufficiently different from the void time. However, applying a lower percentage of acetonitrile would have altered the thinness of the chromatographic peak, leading to a decrease of FUH_2 intensity. The 70% acetonitrile phase was aimed to quickly elute FT, 5-FU and CU that are more strongly retained in the column in an attempt to reduce the total run time.

Multiple reaction monitoring chromatograms of a plasma extract spiked with FT, 5-FU, FUH_2 and CU are presented in Fig. 3 and in Fig. 4 for a patient plasma after FT administration.

3.4. Analytical recovery

The mean analytical recoveries for FUH_2, 5-FU, FT and CU were homogenous and reached 74 ± 5%, 75 ± 7%, 77 ± 6% and 73 ± 6%, respectively.

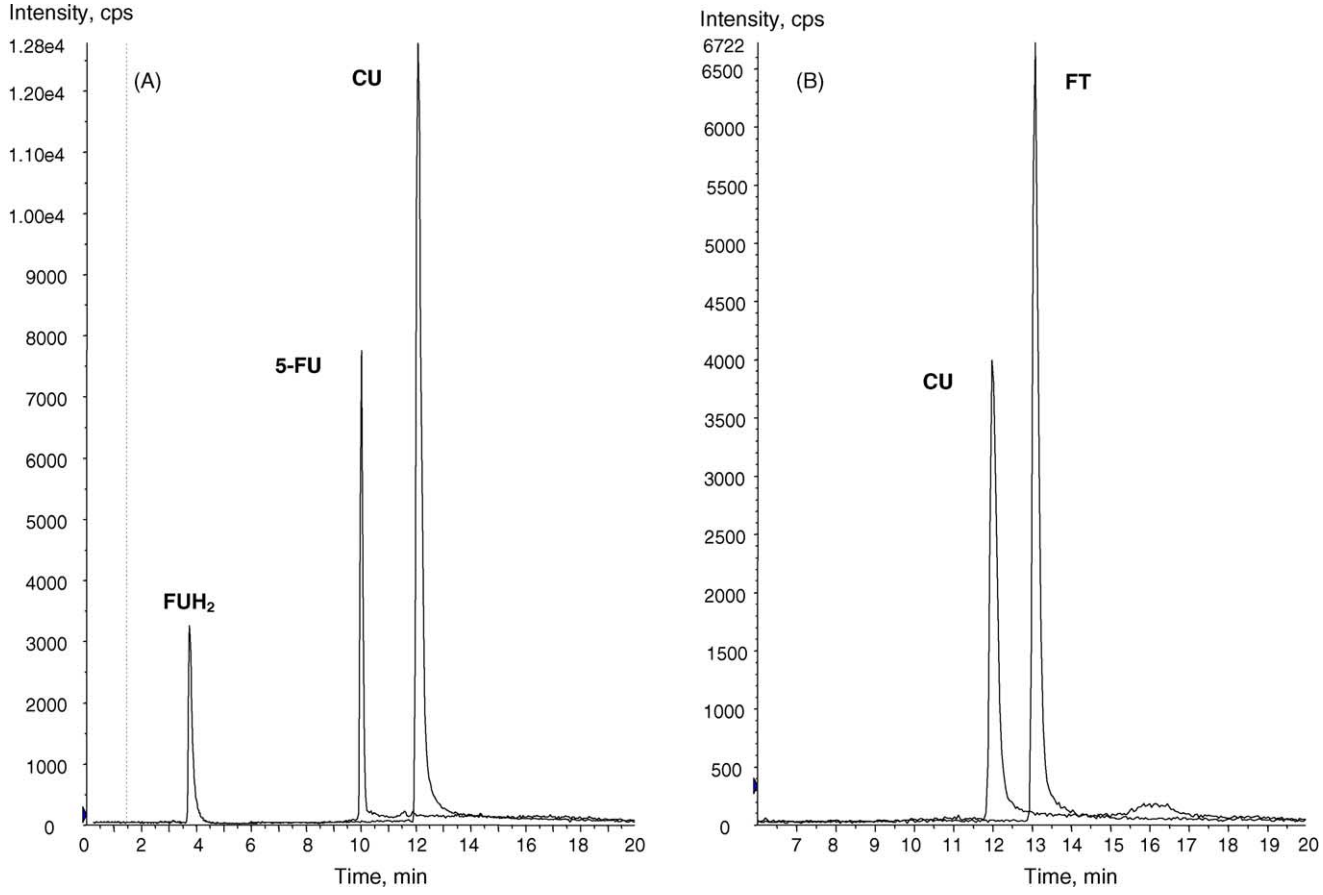

Fig. 3. MRM chromatograms of plasma sample spiked with FUH2, 5-FU, FT and CU at 125, 50, 250 and 125 ng/mL, respectively (A: V_{inj} = 40 μL; B: V_{inj} = 10 μL).

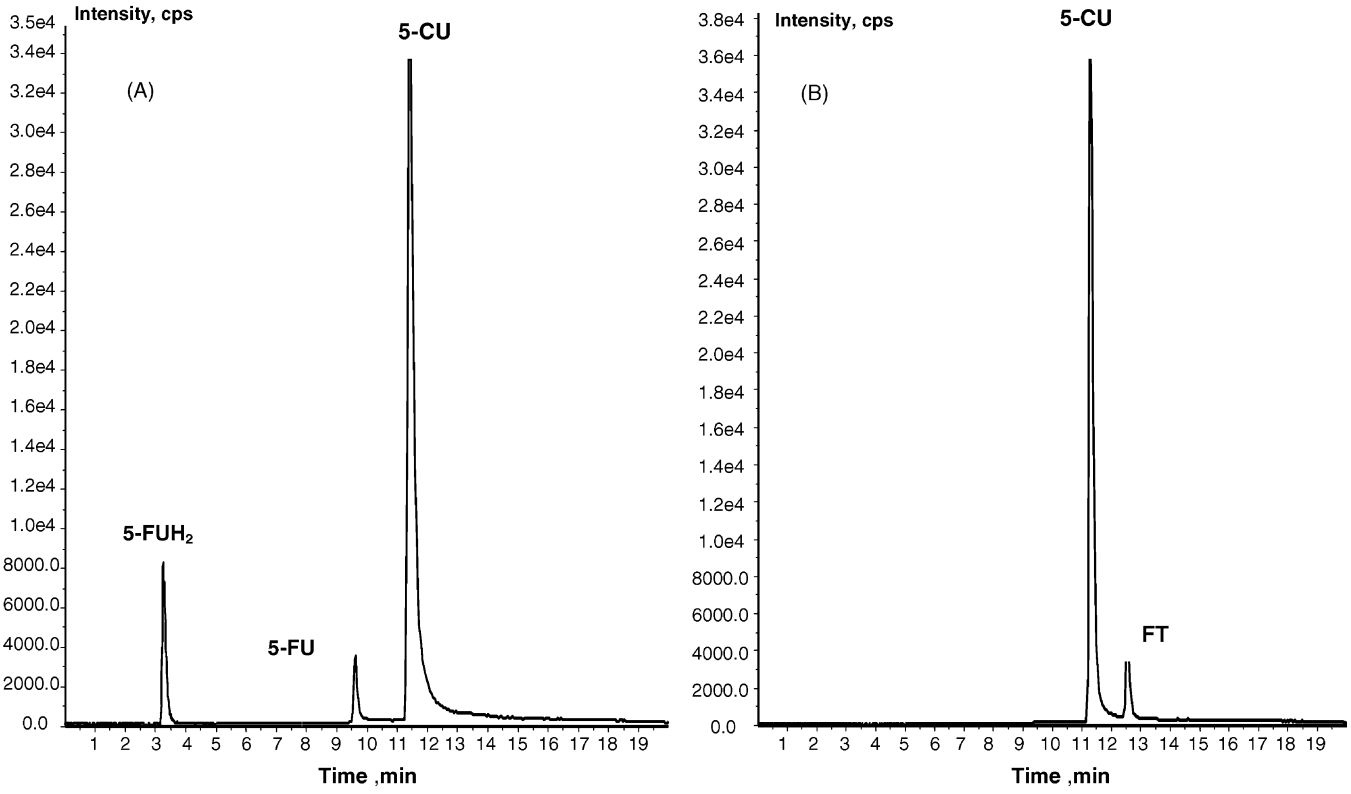

Fig. 4. MRM chromatograms of patient plasma after FT administration (A: V_{inj} = 40 μL; B: V_{inj} = 10 μL).

3.5. Linearity and quantitation limits

The assay validated for linearity of the calibration curves by running five separated freshly prepared plasma standard of: 5–500 ng/mL for 5-FU, 12.5–500 ng/mL for FUH_2, 25–2500 and 2500–25000 ng/mL for FT. The typical equation obtained by least squared regression were $y = 0.0038x + 0.0082$, $y = 0.0002 + 0.0030$, $y = 0.0039 + 0.018$ and $y = 0.0028 + 3.64$ for 5FU, FUH_2 and FT, respectively. Regression coefficients (r^2) were ≥0.9906 for all calibration curves (Table 3).

The LOQ obtained for 5-FU, FUH_2 and FT were 2.5, 12.5 and 6.25 ng/mL, respectively.

3.6. Precision and accuracy

The precision and accuracy were determined with five samples per concentration. All the values are presented in Table 4. The within-day precision (R.S.D.) varied between 3.5 and 6.6% for FUH_2 and between 0.7 and 3.4% for 5-FU. For FT, the R.S.D. was between 1.0 and 3.4% for lower concentrations and between 1.0 and 4.0% for higher concentrations. The between-day precision was found between 1.3 and 4.3% for FUH_2 and between 1.0 and 6.3% for 5-FU. For FT, between-day precision was similar for both concentration ranges and was always between 1.1 and 3.6%.

The accuracy, expressed as the ratio of compound added to that measured, remained in the range 0.2–7.5% for FUH_2 and 5-FU and 0.1–4.0% for FT.

4. Discussion

Because 25–30% of patients develop grade III-IV toxic side effects when treated with 5-FU, oral fluoropyrimidines with lower toxicity profile have been developed. Among the newer fluoropyrimidines, UFT has been studied the most extensively. Several analytical methods have been developed to quantify FT alone or simultaneously with 5-FU in plasma samples [15–19]. However, they are often complicated, with

Table 3
Validation data of linear regression analysis (n = 5)

Parameter	Slope: mean (CV%)	Intercept: mean (CV%)	Correlation coefficient: mean (range)
5FU	0.0038 (1.03)	0.0082 (2.13)	0.09987 (0.9961–0.9997)
FUH_2	0.00033 (1.7)	0.0030 (2.59)	0.9974 (0.9915–0.9999)
FT (25–2500 ng/mL)	0.0039 (1.04)	0.018 (1.27)	0.9979 (0.9906-1)
FT (2500–25,000 ng/mL)	0.0028 (1.02)	3.64 (1.09)	0.9989 (0.9972–0.9999)

Table 4
Precision and accuracy of the method (FT (1) and FT (2) correspond to the lower and higher concentrations of FT, respectively)

Concentration (ng/mL)	Within-day			Between-day		
	Mean ± S.D.	R.S.D. (%)	Accuracy (%)	Mean ± S.D.	R.S.D. (%)	Accuracy (%)
FUH_2						
12.5	12.6 ± 0.5	4.4	0.6	12.5 ± 0.5	4.3	0.3
25	23.6 ± 1.6	6.6	5.5	25.3 ± 0.7	3.0	1.1
50	48.5 ± 2.4	4.9	2.9	50.7 ± 1.6	3.2	1.5
125	119.4 ± 4.8	4.0	4.5	126.0 ± 3.2	2.5	0.8
250	249.6 ± 9.6	3.8	0.2	248.8 ± 5.9	2.4	0.5
500	498.4 ± 17.3	3.5	0.3	501.4 ± 6.7	1.3	0.3
5-FU						
5	5.2 ± 0.1	2.3	4.4	5.4 ± 0.2	3.5	7.5
12.5	13.4 ± 0.3	2.1	5.1	12.7 ± 0.8	6.3	1.9
25	25.7 ± 0.2	0.7	2.7	25.5 ± 0.8	3.3	2.2
50	53.7 ± 1.8	3.4	7.4	49.6 ± 1.7	3.4	0.9
125	124.0 ± 2.0	1.6	0.8	126.0 ± 3.4	2.7	0.8
250	243.4 ± 7.3	3.0	2.6	245.8 ± 2.6	1.0	1.7
500	478.4 ± 4.0	0.8	4.3	499.0 ± 17.6	3.5	0.2
FT (1)						
25	24.4 ± 0.4	1.7	2.2	24.5 ± 0.3	1.3	2.2
50	48.5 ± 1.1	2.4	3.0	51.2 ± 1.8	3.5	2.4
250	245.2 ± 3.9	1.6	1.9	253.2 ± 2.8	1.1	1.3
500	502.0 ± 8.6	1.7	0.4	500.6 ± 8.7	1.7	0.1
1000	1011.4 ± 34.0	3.4	1.1	1008.2 ± 20.3	2.0	0.8
2500	2498.0 ± 24.9	1.0	0.1	2490.0 ± 45.3	1.8	0.4
FT (2)						
2500	2508.0 ± 28.9	1.0	0.3	2494.0 ± 74.0	3.0	0.2
5000	5056.0 ± 97.4	1.9	1.1	5035.0 ± 71.4	1.4	0.7
10000	10118.0 ± 112.3	1.1	1.2	10142.0 ± 308.7	3.0	1.4
15000	15140.0 ± 328.6	2.2	0.9	14880.0 ± 370.1	2.5	0.8
25000	25060.0 ± 991.5	4.0	0.2	24920.0 ± 887.1	3.6	0.3

the necessity of derivatization step or complex detection systems. Moreover, none of them allowed the simultaneous determination of FT, 5-FU and FUH_2.

Therefore, we have set up a simple and reliable LC/MS–MS method to detect these three compounds in human plasma. The use of tandem mass spectrometry as detection system, with MRM transitions specific of each compound, allowed a reliable quantitation of FT, 5-FU and FUH_2. Separation was performed on a Hypercarb column, packed with porous graphitic carbon stationary phase, leading to a better retention of polar compounds than other conventional columns. The retention times thus obtained for FUH_2, 5-FU and FT were 3.5, 10.0 and 13.1 min, respectively. Moreover, the presence of acetonitrile in mobile phase (between 12 and 70%) facilitated evaporation in the mass spectrometer source.

Linearity, precision and accuracy were validated in the concentration range studied for each compound. Considering the most recently published methods allowing the determination of FT and 5-FU, sensitivity obtained with our method was similar to those of Matsushima et al. and Chu et al. [16,22] and better than those of Jarugula et al. and Zufia et al. [21,23].

In conclusion, this LC/MS–MS method allows the specific, sensitive and reproducible quantification of FT, 5-FU and FUH_2 in human plasma. This method can be applied to pharmacokinetic studies in patients treated with oral UFT or S1. Moreover, determination of FT, 5-FU and FUH_2 plasma concentrations allows an evaluation of CYP2A6 and DPD activities, which exhibit wide interindividual variability [3–5,12–14]. Calculating 5-FU/FT ratio could help to predict efficacy of the transformation of FT to 5-FU, whereas FUH_2/5-FU ratio could give information on 5-FU elimination. Both ratios could thus be indicative of the quantity of 5-FU available for anabolism. Individual drug dose adjustment could then be proposed at each course of treatment according to the patient's metabolic capacities. This would lead to an optimization of the treatment by increasing its efficacy and reducing toxicity.

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References

[1] Y.M. El Sayed, W. Sadee, Cancer Res. 43 (1983) 4039.
[2] Y.M. El Sayed, W. Sadee, Biochem. Pharmacol. 31 (1982) 3006.
[3] R. Pearce, D. Greenway, A. Parkinson, Arch. Biochem. Biophys. 298 (1992) 211.

[4] T. Shimada, H. Yamazaki, F.P. Guengerich, Xenobiotica 26 (1996) 395.
[5] E.S. Messina, R.F. Tyndale, E.M. Sellers, Pharmacol. Exp. Ther. 282 (1997) 1608.
[6] M. Nakajima, S. Yamagishi, H. Yamamoto, T. Yamamoto, Y. Kuroiwa, T. Yokoi, Clin. Pharmacol. Ther. 67 (2000) 57.
[7] M. Nakajima, J.T. Kwon, N. Tanaka, T. Zenta, Y. Yamamoto, H. Yamamoto, H. Yamazaki, T. Yamamoto, Y. Kuroiwa, T. Yokoi, Clin. Pharmacol. Ther. 69 (2001) 72.
[8] M. Oscarson, R.A. McLellan, H. Gullsten, J.A. Agundez, J. Benitez, A. Rautio, H. Raunio, O. Pelkonen, M. Ingelman-Sundberg, FEBS Lett. 460 (1999) 321.
[9] K. Kitagawa, N. Kunugita, M. Kitagawa, T. Kawamoto, J. Biol. Chem. 276 (2001) 17830.
[10] C. Xu, Y.S. Rao, B. Xu, E. Hoffmann, J. Jones, E.M. Sellers, R.F. Tyndale, Biochem. Biophys. Res. Commun. 290 (2002) 318.
[11] S. Daigo, Y. Takahashi, M. Fujieda, N. Ariyoshi, H. Yamazaki, W. Koizumi, S. Tanabe, K. Saigenji, S. Nagayama, K. Ikeda, Y. Nishioka, T. Kamataki, Pharmacogenetics 12 (2002) 299.
[12] R.A. Fleming, G.A. Milano, M.H. Gaspard, P.J. Bargnoux, A. Thyss, R. Plagne, N. Renee, M. Schneider, F. Demard, Eur. J. Cancer 29A (1993) 740.
[13] Z. Lu, R. Zhang, R.B. Diasio, Cancer Res. 53 (1993) 5433.
[14] M.C. Etienne, J.L. Lagrange, O. Dassonville, R. Fleming, A. Thyss, N. Renee, M. Schneider, F. Demard, G. Milano, J. Clin. Oncol. 12 (1994) 2248.
[15] T. Marunaka, Y. Umeno, K. Yoshida, M. Nagamachi, Y. Minami, S. Fujii, J. Pharm. Sci. 69 (1980) 1296.
[16] E. Matsushima, K. Yoshida, R. Kitamura, K. Yoshida, J. Chromatogr. B Biomed. Sci. Appl. 691 (1997) 95.
[17] E.B. Hills, V.C. Godefroi, I.A. O'Leary, M. Burke, D. Andrzejewski, W. Brukwinski, J.P. Horwitz, J. Pharm. Sci. 66 (1977) 1497.
[18] N. Kawabata, S. Sugiyama, T. Kuwamura, Y. Odaka, T. Satoh, J. Pharm. Sci. 72 (1983) 1162.
[19] A.T. Wu, H.J. Schwandt, C. Finn, W. Sadee, Res. Commun. Chem. Pathol. Pharmacol. 14 (1976) 89.
[20] M. Iwamoto, S. Yoshida, S. Hirose, J. Chromatogr. 310 (1984) 151.
[21] V.R. Jarugula, F.D. Boudinot, J. Chromatogr. B Biomed. Appl. 677 (1996) 199.
[22] D. Chu, J. Gu, W. Liu, F.J. Paul, Q. Dong, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 795 (2003) 377.
[23] L. Zufia, A. Aldaz, C. Castellanos, J. Giraldez, Ther. Drug Monit. 25 (2003) 221.
[24] E. Gamelin, M. Boisdron-Celle, F. Larra, J. Robert, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 3155.